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Acclimation response of spring wheat in a free-air CO₂ enrichment (FACE) atmosphere with variable soil nitrogen regimes. 2. Net assimilation and stomatal conductance of leaves

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Abstract

Atmospheric CO₂ concentration continues to rise. It is important, therefore, to determine what acclimatory changes will occur within the photosynthetic apparatus of wheat (*Triticum aestivum* L. cv. Yecora Rojo) grown in a future high-CO₂ world at ample and limited soil N contents. Wheat was grown in an open field exposed to the CO₂ concentration of ambient air [370 μmol (CO₂) mol⁻¹; Control] and air enriched to ~200 μmol (CO₂) mol⁻¹ above ambient using a Free-Air CO₂ Enrichment (FACE) apparatus (main plot). A High (35 g m⁻²) or Low (7 and 1.5 g m⁻² for 1996 and 1997, respectively) level of N was applied to each half of the main CO₂ treatment plots (split-plot). Under High-N, FACE reduced stomatal conductance (g_s) by 30% at mid-morning (2 h prior to solar noon), 36% at midday (solar noon) and 27% at mid-afternoon (2.5 h after solar noon), whereas under Low-N, g_s was reduced by as much as 31% at mid-morning, 44% at midday and 28% at mid-afternoon compared with Control. But, no significant CO₂ × N interaction effects occurred. Across seasons and growth stages, daily accumulation of carbon (A') was 27% greater in FACE than Control. High-N increased A' by 18% compared with Low-N. In contrast to results for g_s , however, significant CO₂ × N interaction effects occurred because FACE increased A' by 30% at High-N, but by only 23% at Low-N. FACE enhanced the seasonal accumulation of carbon (A'') by 29% during 1996 (moderate N-stress), but by only 21% during 1997 (severe N-stress). These results support the premise that in a future high-CO₂ world an acclimatory (*down-regulation*) response in the photosynthetic apparatus of field-grown wheat is anticipated. They also demonstrate, however, that the stimulatory effect of a rise in atmospheric CO₂ on carbon gain in wheat can be maintained if nutrients such as nitrogen are in ample supply.

Abbreviations: A – instantaneous leaf net assimilation rate [μmol (CO₂) m⁻² s⁻¹]; A' – daily integral of net leaf carbon accumulation [g(C) m⁻² d⁻¹]; A'' – seasonal integral of net leaf carbon accumulation [g(C) m⁻² yr⁻¹];

ANOVA – analysis of variance; C_a – atmospheric carbon dioxide concentration; C_i – internal carbon dioxide concentration; CL – Control-Low; CH – Control-High; CO_2 – carbon dioxide effect in ANOVA; DAP – day after planting; FACE – free-air- CO_2 -enrichment; FL – FACE-Low; FH – FACE-High; g_s – leaf stomatal conductance [$\text{mol (H}_2\text{O) m}^{-2} \text{ s}^{-1}$]; GS – growth stage in ANOVA; LHC II – light harvesting complex; N – nitrogen effect in ANOVA; TOD – time of day [mid-morning (2.5 h prior to solar noon), midday (solar noon), mid-afternoon (2.5 h after solar noon)] effect in ANOVA; $V_{c,\max}$ – maximum Ribulose-1,5-bisphosphate saturated rate of carboxylation *in vivo*; YR – year effect in ANOVA

Introduction

The report of the IPCC (Intergovernmental Panel on Climate Change 1996) projects that atmospheric CO_2 is rising. A rise in atmospheric CO_2 concentration will directly affect diffusion conductance of CO_2 and H_2O through stomata (Morrison 1987, 1993, 1998). However, this effect may be different for wheat (*Triticum aestivum* L.) grown with adequate water, but limited N, compared with that observed for wheat grown with adequate N, but limited water (Sionit et al. 1980b, 1981b; Wall et al. 1994, 1995; Garcia et al. 1998). The direct effect of elevated CO_2 on reducing stomatal conductance (g_s) and subsequent net assimilation rate (A) may differ because acclimatory changes (*down-regulation*) within the photosynthetic apparatus can occur in response to reduced N supply (Sage 1994). Hence, we need to separate the relative effects that a rise in atmospheric CO_2 concentration will have on both diffusion processes mediated by stomata and biochemical processes mediated by the amount and activity of Rubisco in wheat when water is unlimited and nitrogen is in both ample and limited supply.

Under nonlimiting water and nutrient conditions, a relatively constant internal CO_2 concentration (C_i) of between 20 and 30% below that of atmospheric is observed in C_3 plants (Wong et al. 1979; Von Caemmerer and Farquhar 1981; Farquhar and Wong 1984; Ball et al. 1987; Mott 1988). But, any change in the response of stomata because of elevated CO_2 and limited N supply, may alter plant water relations and as a consequence alter the relationship between C_i and atmospheric CO_2 concentration (C_i/C_a). Any decrease in carboxylation capacity or reduction in g_s because of elevated CO_2 and N stress would tend to increase C_i/C_a (Araus et al. 1986b; Loreto et al. 1994; Grossman-Clarke et al. 1999). Thus, as the CO_2 concentration of the atmosphere increases, C_i/C_a may be a good indicator of any acclimation response of the photosynthetic apparatus to either water or N stress, or their interaction with other environmental constraints (Sage 1994).

Short-term elevation of atmospheric CO_2 concentration stimulates carbon gain in C_3 plants because of an increased substrate availability at the primary carboxylating enzyme (Rubisco) (Long and Drake 1991, 1992; Woodrow 1994; Sage 1994; Van Oosten and Besford 1994; Webber et al. 1995) and suppressed photorespiration (Sharkey 1988; Bowes 1991; Stitt 1991). However, under limited N supply, a reduction in both carboxylation efficiency and maximum rate of carboxylation ($V_{c,\max}$) (Webber et al. 1995), along with a reduction in sink strength (Rogers et al. 1993, 1996) at elevated CO_2 may cause a *down-regulation* of photosynthetic proteins (Van Oosten and Besford 1994; Van Oosten et al. 1994). However, reports on the effect of elevated CO_2 on carboxylation capacity ($V_{c,\max}$, Rubisco content and activity) in wheat have been mixed because Delgado et al. (1994) found no change regardless of N supply, whereas in young plants grown with ample N supply and warm temperatures an *up-regulation* of photosynthetic capacity was shown by Habash et al. (1995). In contrast, Barnes et al. (1995) reported a *down-regulation* in wheat. Rowland-Bamford et al. (1991) reported *down-regulation* in rice (*Oryza sativa* L.) grown in sunlit controlled-environment cabinets as the CO_2 concentration of the air increased from 160 to 900 $\mu\text{mol (CO}_2\text{) mol}^{-1}$. They suggested that *down-regulation* involved modulation of both activity and amount of Rubisco protein in the leaf because Rubisco content and specific activity declined significantly as atmospheric CO_2 concentration increased. Despite mixed results, most chamber-based studies suggest that a reduction in carboxylation efficiency occurs even under ample N (McKee and Woodward 1994a,b; McKee et al. 1995). However, most of these studies have utilized plants grown in controlled-environment or open-top chambers where an increase in atmospheric CO_2 concentration has been accompanied by significant alteration of microclimate (Kimball et al. 1997), and especially where in many cases artificial restrictions of rooting volume or cold soil temperatures may have imposed a lim-

itation (Arp 1991, 1993; Sage 1994; Oechel et al. 1994).

Despite any alteration in carboxylation capacity, wheat grown with elevated CO_2 and limited N supply may still have enough of an increase in assimilate supply that will dilute tissue N content (Rogers et al. 1993, 1996; Lou et al. 1994; Drake et al. 1997). Undoubtedly, reduction in leaf tissue N concentration will reduce Rubisco content and activity (Sage 1994; Webber et al. 1995; Vu et al. 1997, 1998; Adam et al. 1997, 2000) that will ultimately cause a reduction in carboxylation capacity (Evans 1983; Pettersson and McDonald 1994). In a FACE wheat experiment, Nie et al. (1995a,b) reported no reduction in carboxylation efficiency, light harvesting complex (LHC II), or N content for uppermost expanded sunlit leaves of wheat grown with ample N and water supply. In a companion study, Osborne et al. (1998) reported that reductions in A were not observed for the uppermost expanded sunlit leaf, but elevated CO_2 decreased carboxylation efficiency for shaded leaves lower in the canopy. Carboxylation efficiency was also found to be dependent on leaf position and ontogeny for spring wheat grown in open-topped-chambers (Mitchell et al. 1998). In a controlled-environment study, Lawlor et al. (1993) demonstrated that the affect of elevated CO_2 on photosynthetic capacity was dependent on environment constraints and ontogeny of the plant. Leaves that emerged later in the ontogeny of the wheat plant had a progressive decrease in photosynthetic capacity. Levels of photon flux density have also been shown to affect the interaction between photosynthetic capacity and CO_2 level, because an acclimation response decreased photosynthetic capacity in a shade adapted forest under story plant, Indiana strawberry (*Duchesnea indica*) (Osborne et al. 1997). This decrease was accompanied by a 22% increase in maximum quantum efficiency of net photosynthesis and a lower light compensation point. This response enabled this herbaceous perennial grown under elevated CO_2 to have positive net carbon uptake even when light levels were insufficient for the ambient grown plants. In the lower leaves of wheat during vegetative growth, N was reallocated from Rubisco to LHC II, typical of shade-acclimated leaves (Evans 1993). During reproductive growth, N was reallocated for grain development (Simpson et al. 1983; Simpson 1992; Fisher 1993). Osborne et al. (1998) reported that photosynthetic acclimation and the redistribution of N at elevated CO_2 was dependent on leaf age, vertical position within the canopy and development stage.

Furthermore, they postulated that reduced carboxylation capacity was due to a CO_2 -based N dilution effect (Estiarte et al. 1999; Sinclair et al. 2000). Acclimation may be interpreted as an increase in the efficiency of N usage, i.e. a balance between N allocated for growth (Coleman et al. 1993; Rogers et al. 1993, 1996; Sage 1994; Drake et al. 1997), energy transduction (Osborne et al. 1997) and carboxylating capacity (Woodrow 1994; Webber et al. 1995). Nevertheless, under elevated CO_2 and more extreme N deficits, a significant reduction in photosynthetic capacity might occur even for the uppermost expanded sunlit leaf. Such an acclimatory response may reduce assimilate supply independent of any direct effect of elevated CO_2 on stomatal response.

Results from previous free-air CO_2 enrichment (FACE) wheat experiments, where water was limiting and N was nonlimiting, indicated that interactive effects of elevated CO_2 and water stress affected stomata mediated diffusion processes (Wall et al. 1994, 1995; Garcia et al. 1998). Carboxylation efficiency, however, was not affected until N became limiting during senescence (Nie et al. 1995a,b; Osborne et al. 1998). Nevertheless, the interactive effects of elevated CO_2 and N stress on A , g_s and C_i/C_a are still unclear. In light of these observations, it is reasonable to make the following hypotheses: (1) regardless of N supply, a rise in atmospheric CO_2 concentration will reduce g_s ; (2) but, a significant increase in A because of elevated CO_2 will only be observed when N is not severely limiting; (3) with ample N supply, a balance between N needed to maintain photosynthetic capacity and that needed for growth will occur; (4) but, in order to maintain growth under more severe N stress, a greater reallocation of N from the photosynthetic apparatus to structural tissue will occur; (5) any reallocation of N away from the photosynthetic apparatus under limited N supply, to maintain growth, constitutes an acclimatory response.

The rationale for this study was to provide evidence for any acclimatory response in wheat leaves from the biochemical (Adam et al. 1997, 2000), to the individual leaf (reported herein), to the whole-canopy (Brooks et al. 2000) level. This paper, the second in a series of three, reports results on the interactive effects of elevated CO_2 and N levels on carbon gain of wheat leaves grown in an open field. Differences in carbon gain within a day and across days and growing seasons were used to make inferences about acclimatory responses in the photosynthetic apparatus to elevated CO_2 and limited N supply.

Materials and methods

Experimental site and CO₂ and H₂O treatment description

Full details of the experimental site, cultivation, irrigation, fertilization, other cultural practices and the FACE apparatus have been reported elsewhere (Hendrey 1993; Wall and Kimball 1993; Pinter et al. 1996; Kimball et al. 1999). Briefly, a two-year study on hard red spring wheat (cv. Yecora Rojo) was conducted in an open field at the University of Arizona, Maricopa Agricultural Center, located 50 km south of Phoenix, Arizona (33.1° N, 112.0° W). Wheat seeds were sown into flat beds in east-west rows parallel to the irrigation tubing (0.5 m tube spacing, 0.3 m emitter spacing, 0.2 m depth) on 14–15 December 1995 and 15 December 1996. Seeding rates were 109 kg ha⁻¹ (~236 seeds m⁻²; planting density of 189 plants m⁻²) during 1995 and 111 kg ha⁻¹ (~252 seeds m⁻²; planting density of 194 plants m⁻²) during 1996 (50% emergence occurred on 01 January 1996, and the crop was harvested on 29–30 May 1996; 50% emergence occurred on 30 December 1996, and the crop was harvested on 28–29 May 1997). Following sowing, a FACE apparatus (Hendrey 1993) was erected on site to enrich the CO₂ concentration of ambient air (ca. 370 µmol mol⁻¹) above the wheat crop by approximately 200 µmol mol⁻¹ (main plot). Enrichment began within a few days of 50% emergence (01 January 1996; 03 January 1997) for 24 h until physiological maturity (15 May 1996; 12 May 1997). The average daytime CO₂ concentrations in the FACE and Control plots were 548 and 363 µmol mol⁻¹, respectively, while the nighttime values were 598 and 363 µmol mol⁻¹. Seasonal average concentrations were within 0.5 µmol mol⁻¹ of the set point and 93% of the 1-min. averages were within 10% of the set point. On average, the contamination of the Control plots from the FACE plots was <15 µmol mol⁻¹ (Kimball et al. 1999).

During 1996, soil had 8.0 g N m⁻² of ammonium plus nitrate in the top 0.9 m profile. Two N treatments were applied as a strip-plot because each strip across the field contained the same N level. The High-N strip-plot received 5.0, 12.5, 12.5 and 5.0 g N m⁻² from ammonium nitrate through the sub-surface drip-tape irrigation system on 30 January (5-leaf), 22 February (mid-tillering), 30 March (anthesis) and 18 April (early grain fill), respectively, for a total amount of 35 g N m⁻² during 1996. The Low-N treatment re-

ceived 1.5, 3.0, 2.5 and 0 g N m⁻² on irrigation dates given above for a total of 7.0 g N m⁻². Nitrate in the irrigation water added 3.3 and 3.0 g N m⁻² to the High- and Low-N plots, respectively.

During 1997, soil had 14.5 g N m⁻² as ammonium plus nitrate in the top 0.9 m profile. The High-N strip-plot received 5.0, 12.5, 12.5, and 5.0 g N m⁻² from ammonium nitrate through a sub-surface drip-tape irrigation system on 30 January (5-leaf), 5 March (mid-tillering), 18 March (anthesis) and 22 April (early grain fill), respectively, for a total amount of 35 g N m⁻² during 1997. In the Low-N treatment, preplant concentration of ammonium plus nitrate was about 6.9 g N m⁻². The Low-N treatment received 0.5, 0.5, 0.5 and 0 g N m⁻² on irrigation dates given above for a total of 1.5 g N m⁻². Nitrate in the irrigation water added a further 4.3 and 3.8 g N m⁻² to the High- and Low-N plots, respectively.

The combination of CO₂ and N levels gave four treatments consisting of Control-Low (CL), FACE-Low (FL), Control-High (CH) and FACE-High (FH). Although actual irrigation dates differed between 1996 and 1997, N application amounts and timing with respect to growth stage were similar across years. In order to establish a more severe N stress during 1997 compared with 1996, however, the Low-N treatment received 1.5 g N m⁻² (severe N stress) rather than 7 g N m⁻² (moderate N stress). Furthermore, due to the application of N over several growth stages, and due to mineralization processes, the availability of soil N throughout the ontogeny of the crop for both N-limited and unlimited treatments mimicked that observed in natural grassland ecosystems.

Field measurements of leaf gas exchange rates

Dawn to dusk CO₂ and H₂O gas exchange rates were measured on randomly selected fully expanded sunlit leaves with three portable closed-exchange (transient) systems with 0.25 L transparent assimilation cuvettes¹ (Model LI-6200, LI-COR, Inc., Lincoln, Nebraska). Each infrared gas analyzer was calibrated against a gravimetrically prepared mixture of CO₂ in air (± 1% Primary Standard, Matheson Gas Products, Inc., Cucamonga, California), and cuvette humidity sensors were calibrated with a dew-point generator (LI-610, LI-COR Inc., Lincoln, Nebraska) immediately prior to use. Measurements of A [µmol (CO₂) m⁻² s⁻¹] and

¹ Mention of this or any other proprietary product does not imply an endorsement or recommendation by the authors or their institution over products not mentioned.

g_s [$\text{mol}(\text{H}_2\text{O}) \text{m}^{-2} \text{s}^{-1}$] began at a leaf cuvette CO_2 concentration of 370 ± 35 or $550 \pm 35 \mu\text{mol mol}^{-1}$ for Control and FACE, respectively, on the central portion of fully expanded (ligule emerged) upper-canopy sunlit leaves. The leaf cuvette was held in the horizontal position and caution was used not to shade any portion of the leaf. One leaf per row from each of five different rows (repeated measures) was randomly selected in the area of each subplot (Wall and Kimball 1993) designated for net assimilation studies for three replications for High- and Low-N. Because there were three instruments, all three replications were measured simultaneously, thereby minimizing variation in gas exchange measurements because of diurnal changes in meteorological conditions, particularly sun angles and incident photosynthetically active radiation. Individual runs were completed in less than 1.5 h. Three observations were recorded at 10-s intervals (total measurement time, therefore, was approximately 55 s, which minimized leaf cuvette effects on gas exchange rate measurements). The first 10-s measurement interval was discarded from the statistical analysis because the CO_2 concentration in the assimilation cuvette was unstable during that period. Consequently, each mean datum was derived from 5 leaves \times 2 observations \times 3 replications.

Each portable transient gas-exchange system made direct measurements of atmospheric CO_2 concentration (C_a), air humidity and leaf and air temperatures. We calculated leaf A directly, whereas g_s and C_i were calculated as suggested by LI-COR (1990) following the equations of Von Caemmerer and Farquhar (1981). Phenological development (average numerical decimal code across all treatments) was used to group gas exchange parameters (A , g_s and C_i/C_a) by distinctive growth stage as follows: 5-leaf, tillering, stem-elongation/inflorescence emergence, anthesis, soft and hard dough for 1996/1997 (Zadoks 15-91; Zadoks et al. 1974). A' for each treatment was derived by integrating the respective dawn to dusk A . Dawn and dusk times in mountain standard time (MST) were obtained from standard meteorological tables for Maricopa, Arizona, USA, and served as zero response points for the purpose of integration. A'' was derived by integrating A' from 50% emergence until physiological maturity (25% fractional absorbed photosynthetically active radiation). Integrations were performed using the *Area.xfm* trapezoidal integration routine (Sigma Plot, v. 4.01, SPSS Inc., Chicago, Illinois).

Experimental design and statistical design

All ANOVAs were performed using PROC GLM in SAS (Reference manual version 6.0, 1989) to evaluate the CO_2 , N and any $\text{CO}_2 \times \text{N}$ interaction effects on A , g_s and C_i/C_a . All ANOVAs for C_i/C_a were performed on a $\log_{(10)}$ transformation of C_i/C_a . These effects were evaluated based on a strip-split plot experimental design (CO_2 : main-plot; N: split-plot) as described in a companion paper (Kimball et al. 1999). Time of day (TOD) was the third factor in the ANOVA. It was treated as another split in the design to evaluate the effect of CO_2 and N on A and g_s at mid-morning (2.5 h prior to solar noon), midday (solar noon) and mid-afternoon (2.5 h after solar noon). The effects of CO_2 , N and TOD on A and g_s were evaluated across growth stage (GS) as a repeated measure. Hence, the experimental design was a strip-split-split-repeated measure design. Because N application rate for the Low-N treatments was 7 g N m^{-2} during 1996 compared with only 1.5 g N m^{-2} during 1997, a moderate and a severe N treatment existed in 1996 and in 1997, respectively. Comparisons of the effect of CO_2 and N on A'' across years were performed by treating year (YR) as another repeated measure. To avoid pseudo-replication, all ANOVAs were performed on replication means.

Results

Atmospheric and edaphic factors

Planting dates were similar between years. Consequently, trends in solar radiation, air temperature and vapor pressure deficit were also similar. Most days had predominantly clear skies. The maximum solar and daily integral of global solar radiation ranged from 2.2 to $3.6 \text{ MJ m}^{-2} \text{h}^{-1}$ and from 14 to $28 \text{ MJ m}^{-2} \text{d}^{-1}$. A corresponding increase in maximum air temperature from 18 to 34°C occurred in response to an increase in solar radiation. An increase in the evaporative demand imposed on the crop was evident from the increase in midday vapor pressure deficit, which ranged from 1.0 kPa at tillering to just under 5 kPa at hard dough (AZMET; Brown 1987).

Irrigation scheduling maintained soil volumetric water content near field capacity (Kimball et al. 1999; Hunsaker et al. 2000). Consequently, the effects of elevated CO_2 and N availability on gas exchange processes in wheat could be investigated independent of water effects.

Table 1. Source of variance in ANOVA for carbon dioxide [CO_2 : Control compared with FACE ($370:550 \mu\text{mol mol}^{-1}$)], nitrogen [N: Low compared with High ($7:35 \text{ g m}^{-2}$ during 1995–6 and $1.5:35 \text{ g m}^{-2}$ during 1996–7)], time of day (TOD^a), growth stage as repeated measure (GS)^b and replication (R) effects on net assimilation rate (A), stomatal conductance (g_s) and ratio of internal-atmospheric CO_2 concentration (C_i/C_a) of upper most fully expanded sunlit spring wheat leaves during 1996 and 1997

Source ^c	df	A		g_s		C_i/C_a ^d	
		1996	1997	1996	1997	1996	1997
R	2	ns	ns	ns	*	ns	*
CO_2	1	***	***	***	**	*	*
N	1	*	**	ns	*	ns	ns
$\text{CO}_2 \times \text{N}$	1	ns	ns	**	ns	ns	**
TOD	2	***	ns	ns	ns	ns	***
$\text{TOD} \times \text{CO}_2$	2	ns	ns	ns	ns	ns	ns
$\text{TOD} \times \text{N}$	2	ns	ns	ns	ns	ns	ns
$\text{TOD} \times \text{CO}_2 \times \text{N}$	2	ns	ns	ns	ns	ns	ns
GS	4	***	***	**	**	*	ns
$\text{GS} \times \text{CO}_2$	4	*	**	*	ns	ns	ns
$\text{GS} \times \text{N}$	4	***	**	*	*	ns	ns
$\text{GS} \times \text{CO}_2 \times \text{N}$	4	ns	ns	ns	ns	ns	ns
$\text{GS} \times \text{TOD}$	8	***	*	ns	***	ns	***
$\text{GS} \times \text{TOD} \times \text{CO}_2$	8	ns	**	ns	ns	ns	ns
$\text{GS} \times \text{TOD} \times \text{N}$	8	ns	ns	ns	*	ns	ns
$\text{GS} \times \text{TOD} \times \text{CO}_2 \times \text{N}$	8	ns	ns	ns	ns	ns	ns

***, **, * and ns for $P \leq 0.01$, $P \leq 0.05$, $P \leq 0.10$, and not significant, respectively.

^aMid-morning (2.5 h prior to solar noon), midday (solar noon), and mid-afternoon (2.5 h after solar noon).

^bZadoks growth stage corresponds to 5-leaf (DAP 062), tillering (DAP 083), stem-elongation (DAP 103), anthesis (DAP 116), soft dough (DAP 130) and hard dough (DAP 144) during 1996; and, 5-leaf (DAP 051), tillering (DAP 079), inflorescence emergence (DAP 095), anthesis (DAP 115), and hard dough (DAP 137) during 1997 (Zadoks 15-91; Zadoks et al. 1974).

^cSource of variance in ANOVA (error term) include: R, CO_2 ($\text{R} \times \text{CO}_2$); N ($\text{R} \times \text{N}$); $\text{CO}_2 \times \text{N}$ ($\text{R} \times \text{CO}_2 \times \text{N}$); TOD ($\text{R} \times \text{TOD}$); $\text{TOD} \times \text{CO}_2$ ($\text{R} \times \text{TOD} \times \text{CO}_2$); $\text{TOD} \times \text{N}$ ($\text{R} \times \text{TOD} \times \text{N}$); $\text{TOD} \times \text{CO}_2 \times \text{N}$ ($\text{R} \times \text{TOD} \times \text{CO}_2 \times \text{N}$); GS ($\text{R} \times \text{GS}$); $\text{GS} \times \text{CO}_2$ ($\text{R} \times \text{GS} \times \text{CO}_2$); $\text{GS} \times \text{N}$ ($\text{R} \times \text{GS} \times \text{N}$); $\text{GS} \times \text{CO}_2 \times \text{N}$ ($\text{R} \times \text{GS} \times \text{CO}_2 \times \text{N}$); $\text{GS} \times \text{TOD}$ ($\text{R} \times \text{GS} \times \text{TOD}$); $\text{GS} \times \text{TOD} \times \text{CO}_2$ ($\text{R} \times \text{GS} \times \text{TOD} \times \text{CO}_2$); $\text{GS} \times \text{TOD} \times \text{N}$ ($\text{R} \times \text{GS} \times \text{TOD} \times \text{N}$); and $\text{GS} \times \text{TOD} \times \text{CO}_2 \times \text{N}$ ($\text{R} \times \text{GS} \times \text{TOD} \times \text{CO}_2 \times \text{N}$).

^dANOVA on C_i/C_a performed on $\text{Log}_{(10)}$.

Effects of CO_2 and N on mid-morning, midday and mid-afternoon g_s , A, and C_i/C_a

All results from ANOVAs given below will follow a similar order. Higher order interactions will be discussed first, then lower order interactions, then main effects.

A four-way ANOVA (CO_2 , N, TOD, GS) indicated that the main CO_2 effect predominated for A, g_s and C_i/C_a (Table 1). The main N effect on A was more significant than on either g_s or C_i/C_a . During 1996, a significant $\text{CO}_2 \times \text{N}$ interaction did occur for g_s

and for C_i/C_a during 1997. A significant TOD effect was observed for A during 1996 and for C_i/C_a during 1997 (Table 1). Nevertheless, TOD did not interact with either CO_2 or N (nonsignificant $\text{TOD} \times \text{CO}_2$ and $\text{TOD} \times \text{N}$, respectively). Furthermore, no mid-afternoon depression in either A (Figure 1) or g_s (Figure 2) was observed. Significant GS effects occurred as N became more limiting throughout the ontogeny of the crop, particularly for Low- compared with High-N and during years of moderate (1996) compared with severe (1997) N stress. Presumably, significant $\text{GS} \times \text{CO}_2$ and $\text{GS} \times \text{N}$ effects occurred because of di-

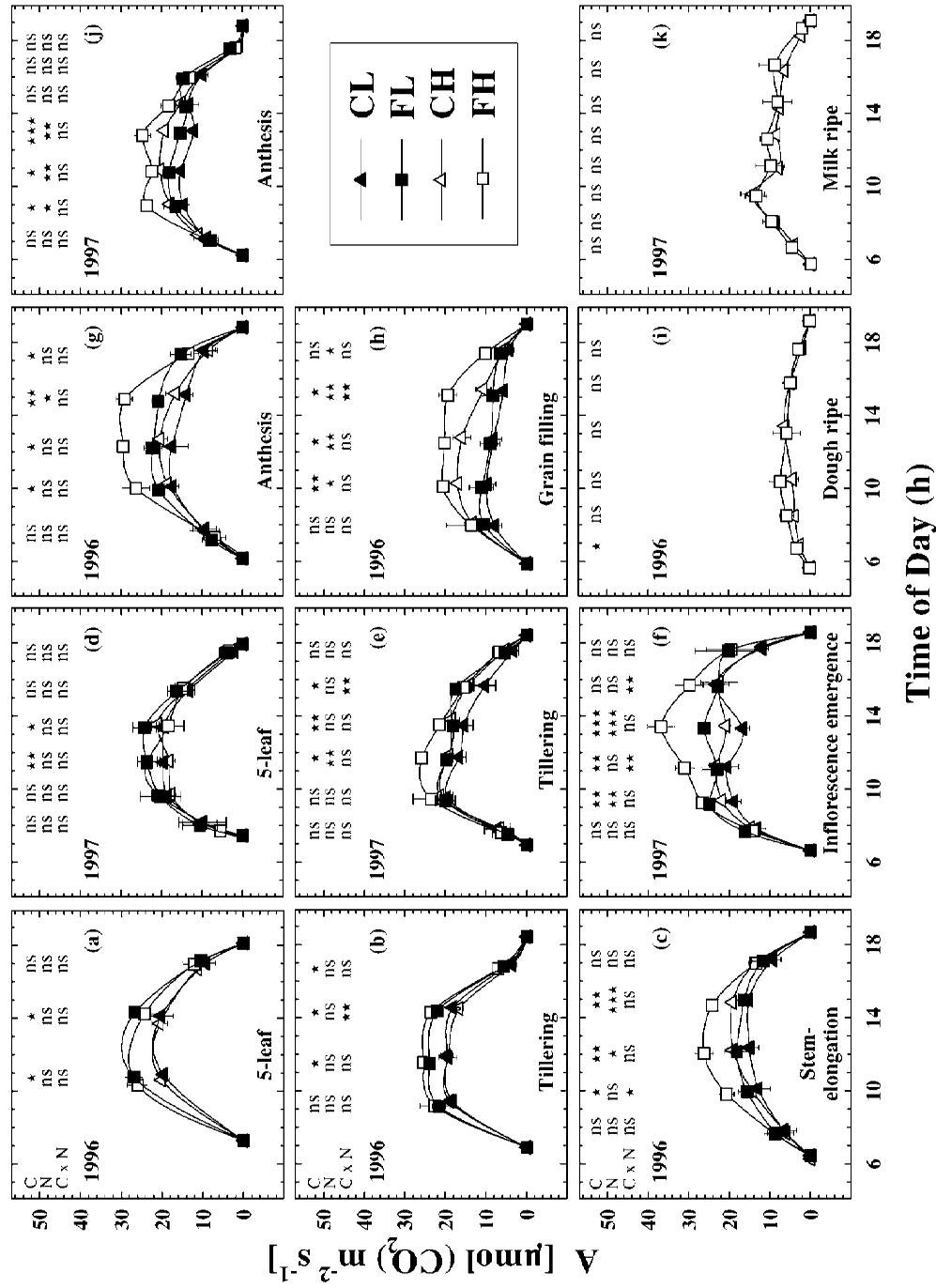


Figure 1. Dawn to dusk trends in net assimilation rate (A) of expanded sunlit wheat [*T. aestivum* (L.) cv. Yecora Rojo] leaves for growth stages given during 1996 (a, b, c, g, h, i) and 1997 (d, e, f, j, k). Treatment legend is as follows: Control-Low (CL); FACE-Low (FL); Control-High (CH); FACE-High (FH). Vertical bar is one standard error from each datum. Percentages in parentheses above each datum indicate relative CO_2 enhancement for Low- and High-N, respectively. Source of variance in ANOVA are carbon dioxide (C) [Control at $370 \mu\text{mol mol}^{-1}$, and FACE at ambient $+200 \mu\text{mol mol}^{-1}$], nitrogen (N) [Low at 7 and 1.5 g N m^{-2} during 1996 and 1997, respectively, and High at 35 g N m^{-2}], and C \times N interaction effects. Significance effects for each growth stage given above each datum as ***, **, *, ns, and ns for $P \leq 0.01$, $P \leq 0.05$, $P \leq 0.10$, not estimated and not significant, respectively.

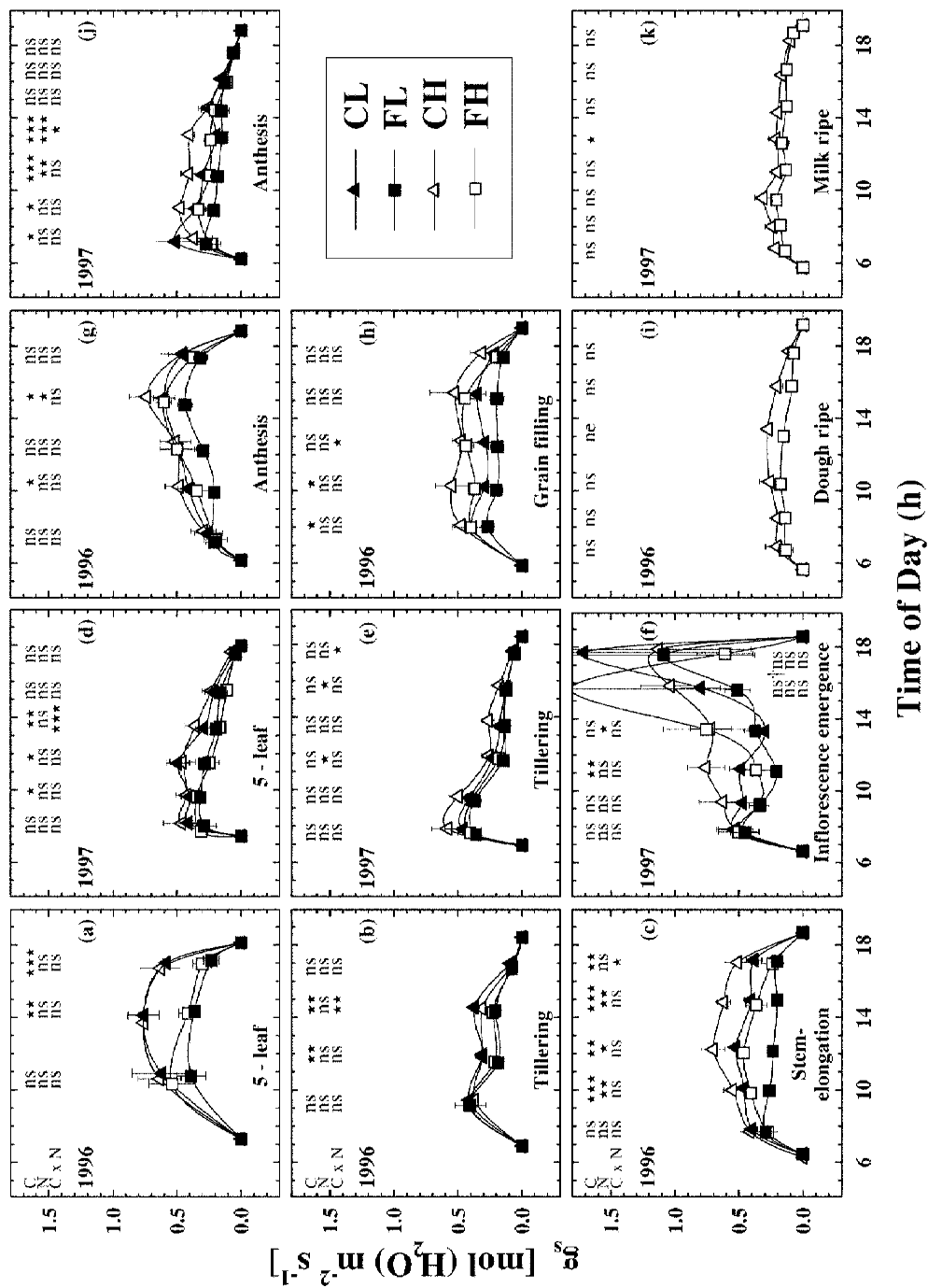


Figure 2. Dawn to dusk trends in stomatal conductance (g_s) of fully expanded sunlit wheat (*T. aestivum* L.) cv. Yecora Rojo leaves for days after planting (DAP) given, which corresponds with growth stages given during 1996 (a, b, c, g, h, i) and 1997 (d, e, f, j, k). Standard errors, percentages in parentheses, symbols in legend, source of variance and results from ANOVA same as described in Figure 1. * Values for g_s for CL off scale for late-afternoon at inflorescence emergence during 1997 (f).

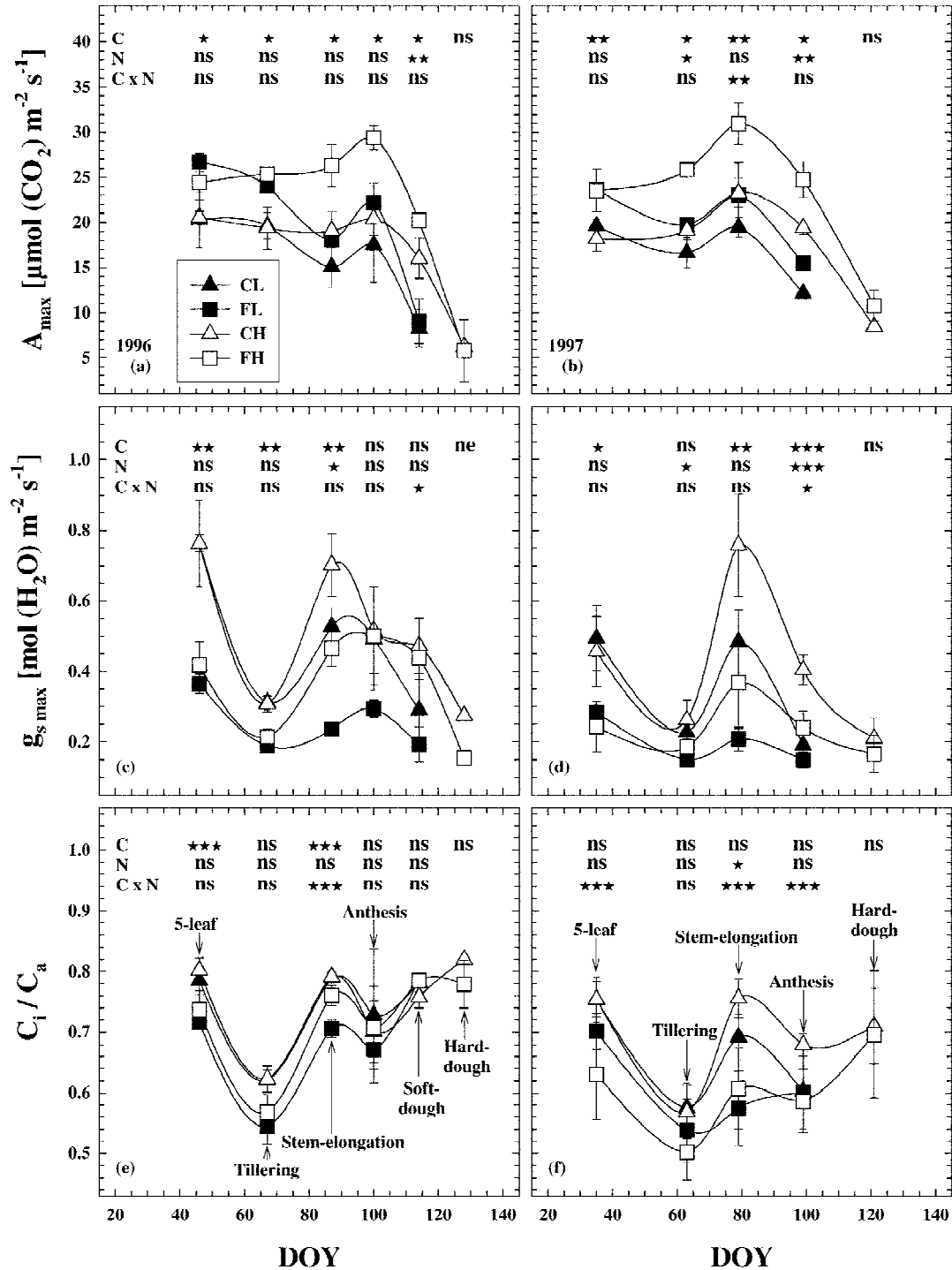


Figure 3. Seasonal midday (solar noon) net assimilation rate (A) (a, b), stomatal conductance (g_s) (c, d), and ratio of internal (C_i) to atmospheric (C_a) CO₂ concentration (C_i/C_a) (e, f) for expanded sunlit wheat [*T. aestivum* (L.) cv. Yecora Rojo] leaves at growth stages given during 1996 (a, c, e) and 1997 (b, d, f). Standard errors, percentages in parentheses, symbols in legend, source of variance and results from ANOVA same as described in Figure 1.

lution of leaf N content during vegetative growth and remobilization of N during reproductive growth.

Throughout a growing season, a consistent treatment order for A was observed (FH>FL>CH>CL; Figures 1, 3a,b). Treatment effects on g_s were somewhat inverted, but generally ranked as CH>CL \cong FH>FL (Figures 2 and 3c,d). Across N, seasons and GS, A was 23, 22 and 31% greater in FACE than Control at mid-morning, midday and mid-afternoon, respectively. Under High-N, FACE increased A by 26, 25 and 36%, whereas under Low-N, FACE increased A by only 21, 22 and 27% at mid-morning, midday and mid-afternoon, respectively. Thus, the increase in the CO₂ effect for High- compared with Low-N was 5% at mid-morning, 3% at midday and 9% at mid-afternoon. Overall, High-N increased A by 15, 21 and 23% at mid-morning, midday and mid-afternoon compared with Low-N, respectively.

Across N, seasons and GS, FACE reduced g_s by 30, 39 and 28% compared with Control at mid-morning, midday and mid-afternoon, respectively (Figure 2). Under High-N, FACE reduced g_s by 30% at mid-morning, 36% at midday and 27% at mid-afternoon, whereas under Low-N g_s was reduced by as much as 31% at mid-morning, 44% at midday and 28% at mid-afternoon. Thus, there was an additional reduction in g_s in FACE compared with Control for Low-compared with High-N of 1% at mid-morning, 8% at midday and 1% at mid-afternoon. These results are consistent with reductions in g_s by 16, 31 and 32% for Low- compared with High-N at mid-morning, midday and mid-afternoon, respectively. The lack of any clear CO₂ \times N interaction effect for g_s suggests that effects of elevated CO₂ and reduced N supply on g_s were additive rather than multiplicative.

During 1996, FACE tended to decrease midday C_i/C_a under moderate N stress (Figure 3e). During 1997, however, when N stress was more severe, effects of elevated CO₂ and N on C_i/C_a were mixed (Figure 3f). Prior to anthesis C_i/C_a was ~ 0.7 , but after anthesis it increased slightly as N became limiting.

Effect of CO₂ and N on daily (A') and seasonal (A'') integrals of A

Results from a three-way ANOVA (CO₂, N, GS) on daily integrals of A (A') were consistent across moderate (1996) and severe (1997) N stress years (Table 2). Across N, YR and GS, A' was 27% greater in FACE (Figure 4) compared with Control. Across CO₂, YR and GS, High-N increased A' by

Table 2. Source of variance in ANOVA for carbon dioxide [CO₂: Control compared with FACE (370:550 $\mu\text{mol mol}^{-1}$)], nitrogen [N: Low compared with High (7.0:35 g m^{-2} during 1995–6 and 1.5:35 g m^{-2} during 1996–7)], growth stage^a (GS) and replication (R) effects for daily integrals of net assimilation rate (A') of uppermost fully expanded sunlit spring wheat leaves during 1996 and 1997

Source ^b	df	A'	
		1996	1997
R	2	ns	ns
CO ₂	1	***	**
N	1	*	**
CO ₂ \times N	1	ns	ns
GS	4	***	***
GS \times CO ₂	4	**	***
GS \times N	4	***	***
GS \times CO ₂ \times N	4	ns	ns

***, **, * and ns for $P \leq 0.01$, $P \leq 0.05$, $P \leq 0.10$, and not significant, respectively.

^aZadoks growth stage corresponds to 5-leaf (DAP 062), tillering (DAP 083), stem-elongation (DAP 103), anthesis (DAP 116), soft dough (DAP 130) and hard dough (DAP 144) during 1996; and, 5-leaf (DAP 051), tillering (DAP 079), inflorescence emergence (DAP 095), anthesis (DAP 115), and hard dough (DAP 137) during 1997 (Zadoks 15–91; Zadoks et al. 1974).

^bSource of variance in ANOVA (error term) include: R, CO₂ (R \times CO₂); N (R \times N); CO₂ \times N (R \times CO₂ \times N); GS (R \times GS); GS \times CO₂ (R \times GS \times CO₂); GS \times N (R \times GS \times N); and GS \times CO₂ \times N (R \times GS \times CO₂ \times N).

18% compared with Low-N. Under High-N, FACE increased A' by 30%, whereas under Low-N it was increased by only 23% (7% reduction in the stimulatory effect of elevated CO₂ on A' for Low- compared with High-N). Furthermore, as N stress became more severe with the ontogeny of the crop, an increase in the likelihood of a significant CO₂ \times N interaction for A' occurred: 5-leaf ($P=0.72$ for 1996; $P=0.01$ for 1997); tillering ($P=0.25$ for 1996; $P=0.99$ for 1997); stem-elongation/inflorescence emergence ($P=0.33$ for 1996; $P=0.03$ for 1997); anthesis ($P=0.25$ for 1996; $P=0.68$ for 1997); soft-dough ($P=0.14$ for 1996). Although a decrease in midday A occurred with GS (Figures 1, 3a,b), presumably because of dilution of tissue N content, an increase in day length caused A' to remain relatively constant for a given treatment throughout the ontogeny of the crop (Figure 4). Nevertheless, significant GS, GS \times CO₂ and GS \times N effects (Table 2) suggest that the compensatory effect of an increase in day length did not totally mitigate the decline in A throughout the growing season. Hence, as N became more limited, the overall stimulatory effect of FACE on A' diminished

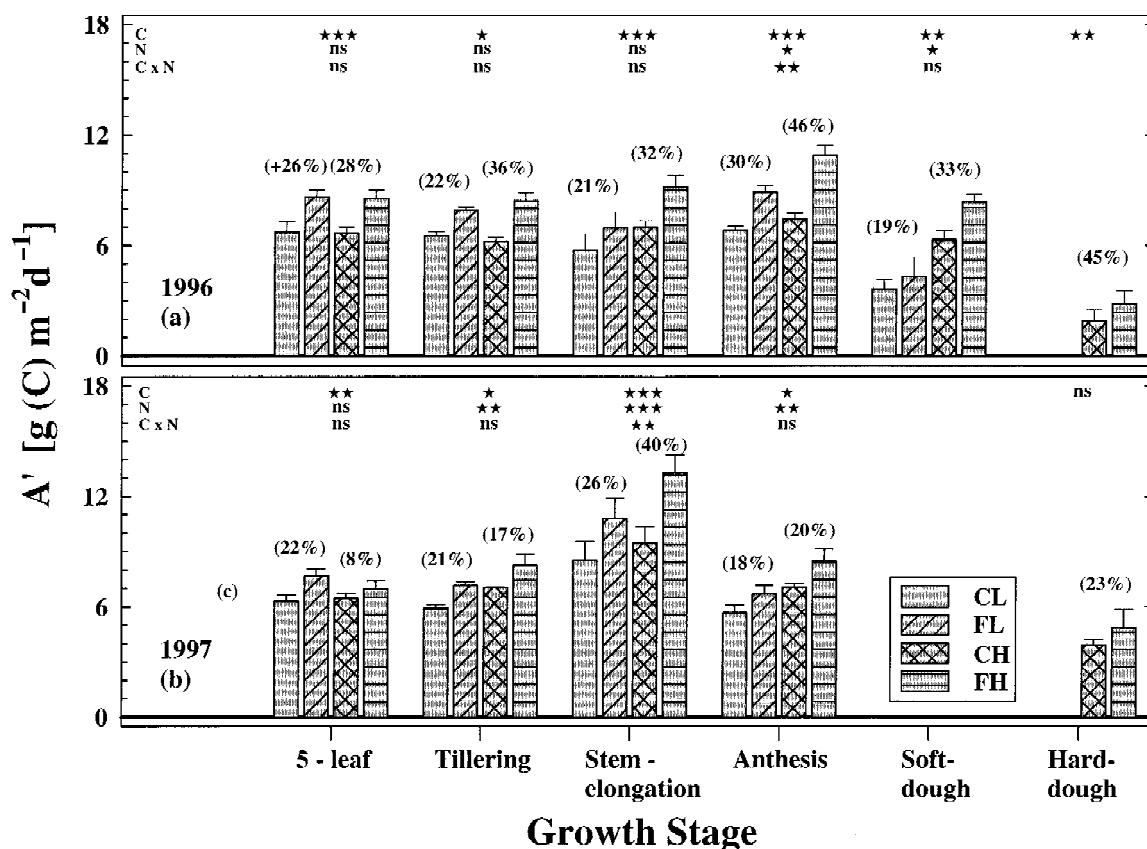


Figure 4. Daily integrals of carbon accumulated (A') for expanded sunlit wheat [*T. aestivum* (L.) cv. Yecora Rojo] leaves at growth stages given during 1996 (a) and 1997 (b). Standard error, percentages in parentheses, symbols in legend, source of variance and results from ANOVA same as described in Figure 1.

(A' was stimulated by -12 , 9 , 13 , 9 and 14% more by FACE than Control for High- compared with Low-N at 5-leaf, tillering, stem-elongation/inflorescence emergence, anthesis and soft-dough stages, respectively, Figure 4).

A three-way ANOVA (CO_2 , N, YR) for A'' indicated that the main CO_2 effect predominated (Figure 5; $P=0.03$). Although the $\text{CO}_2 \times \text{N}$ effect was not significant, FACE stimulated A'' by 28% under High-N and by 23% under Low-N (Figure 5). YR, ($P=0.02$) and $\text{YR} \times \text{CO}_2$ ($P=0.03$) effects occurred because FACE enhanced A'' by 29% during 1996, but by only 21% during 1997. This resulted in an 8% reduction in the stimulatory effect of FACE on A'' under severe (1997) compared with moderate (1996) N stress.

Discussion

For wheat grown with ample N and soil moisture supply, an increase in atmospheric CO_2 concentration is known to reduce stomatal aperture, increase carbon gain, but have only a nominal effect on C_i/C_a in both controlled-environment (Sionit et al. 1980a, 1981a; Morison 1993, 1998; Lawlor et al. 1995) and field studies (Wall et al. 1994, 1995; Garcia et al. 1998). For wheat grown with ample N supply, our field-based results on g_s (Figures 2 and 3c,d) and A (Figures 1 and 3a,b) were in agreement with those reported previously (*accept hypothesis 1 for High N*).

Presumably, N-stress altered anatomical [reduced cell number (Nelson and MacAdam 1989)], architectural (see Figure 5; Brooks et al. 2000), and xeromorphic [increase in cell wall thickening and the volumetric modulus of elasticity (Morgan 1986)] characteristic of individual leaf blades (personnel observa-

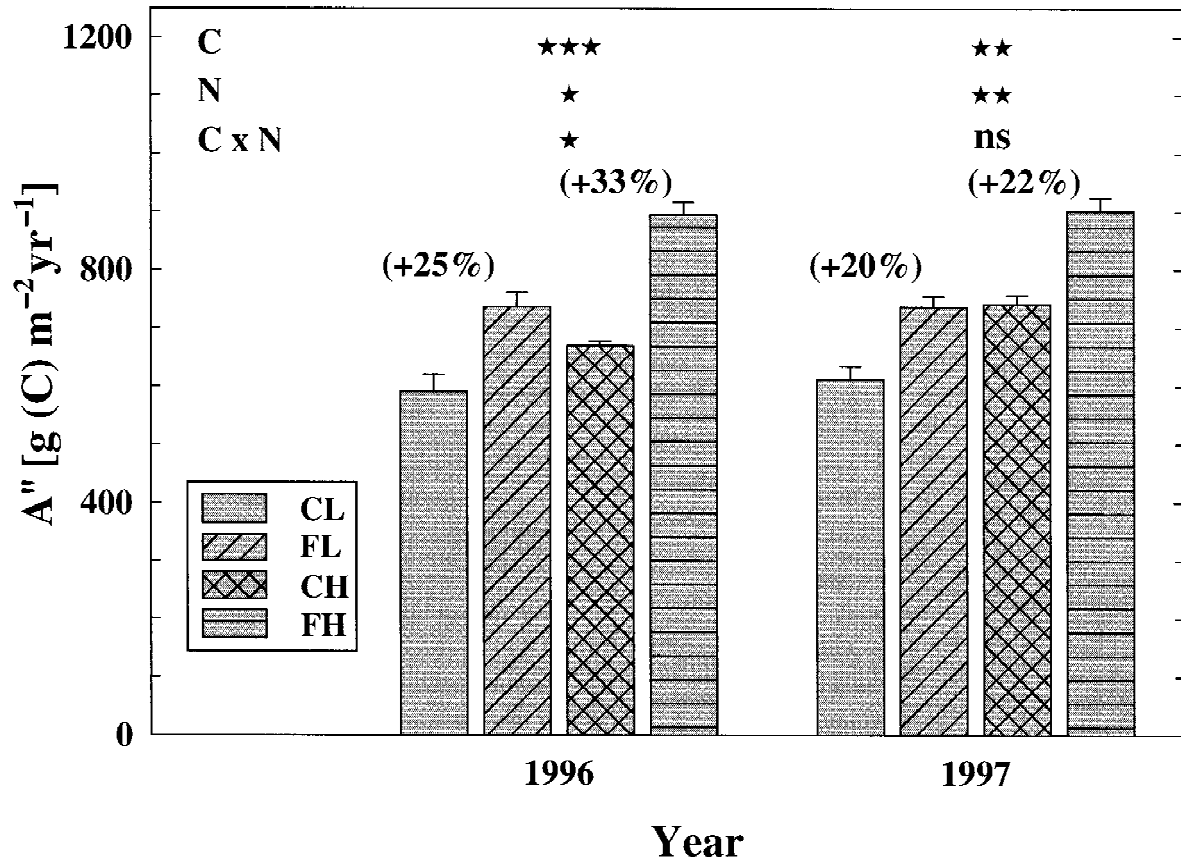


Figure 5. Seasonal integral of carbon accumulated (A'') derived from 50% emergence until physiological maturity for expanded sunlit wheat [*T. aestivum* (L.) cv. Yecora Rojo] leaves during 1996 and 1997. Standard error, percentages in parentheses, symbols in legend, source of variance and results from ANOVA same as described in Figure 1.

tion). But, despite the multiplicative effect of elevated CO_2 and water stress on g_s and C_i/C_a in a prior FACE wheat experiment (Wall et al. 1994, 1995), the effect of elevated CO_2 and N stress on g_s (Figures 2 and 3c,d) and C_i/C_a (Figure 3e,f) appeared to be additive. Environmental constraints, such as water stress, have been shown to cause mid-afternoon depressions in g_s which reduce carbon gain (Sionit et al. 1980b; Kramer 1983; Tenhunen 1984). Mid-afternoon depression in g_s and subsequent carbon gain were observed in the Dry treatment of the FACE wheat $\text{CO}_2 \times$ Irrigation experiment (Wall et al. 1994, 1995), which resulted in significant CO_2 by Irrigation by TOD interaction effect for g_s and C_i/C_a . In contrast, during this study CO_2 and N did not interact with TOD for either A , g_s , or C_i/C_a (nonsignificant $\text{TOD} \times \text{CO}_2$ and $\text{TOD} \times \text{N}$ interaction effects, Table 1). These results suggest that although N stress altered anatomical characteristics towards more xeromorphic traits, these changes

caused only a minor additional reduction in g_s than that caused by elevated CO_2 alone (*accept hypothesis 1 for Low N*) (Figures 2 and 3b,c).

In a companion study, Wechsung et al. (2000) reported that the effects of elevated CO_2 on g_s of ears of wheat was similar regardless of either water or N stress. Perhaps, water or N stress-based adaptations may not occur to the same degree in ears as they apparently did in leaves, because g_s of ears was less responsive to either water or N stress. Nevertheless, elevated CO_2 caused a significant increase in carbon gain in leaves within a day (Figure 1), across days (Figure 4) and seasons (Figure 5). Because N stress only caused an additional 5% reductions in g_s (Figure 2), a proportionately greater reduction in carbon gain for N-stressed wheat grown in elevated CO_2 (Figures 1, 3a,b, 4 and 5) occurred because of a decrease in carboxylation capacity (Adam et al. 2000), rather than any direct effect of N stress on diffusion conductance

of CO₂ into the substomatal cavity (*accept hypothesis 2*).

Reduced plant N concentration has been commonly observed with ontogeny of grasses (Hocking and Meyer 1991a,b; Newton 1991; Conroy 1992; Conroy and Hocking 1993; Coleman et al. 1993; Owensby et al. 1993a). Apparently, an increase in atmospheric CO₂ concentration exacerbates this effect, because an increase in assimilate supply (Figures 1, 3a,b, 4 and 5) and subsequent growth (Pinter et al. 1996) causes a dilution of leaf tissue N concentration (Lue et al. 1994; Drake et al. 1997). Morgan et al. (1994) demonstrated that photosynthetic acclimation occurred for two native shortgrass steppes grasses (*P. smithii*, *B. gracilis*), but that reductions in photosynthetic capacity were related more to reductions in leaf N concentration than to the accumulation of non-structural carbohydrates. In companion studies, Estiarte et al. (1999) and Sinclair et al. (2000) reported significant reductions in whole-canopy leaf tissue N concentration with the ontogeny of wheat that were significantly greater in the elevated CO₂ and Low-N treatment. Reduced plant N concentrations may indicate a reduced N requirement (Conroy 1992; Long et al. 1993). A reduction in leaf tissue N concentration under elevated CO₂ results from an active reallocation of N by plants from photosynthetic proteins to maintain structural growth (Hilbert et al. 1991; Morison and Lawlor 1999). Perhaps, the greater dilution of leaf tissue N concentration that occurred because of elevated CO₂ and Low-N supply can explain the increased likelihood of a significant CO₂ × N interaction effect for A (Figures 1 and 3a,b) and A' (Figure 4) with crop ontogeny. This was most obvious at stem-elongation during 1996 (Figures 1c and 4a) and inflorescence emergence during 1997 (Figures 1f and 4b), and can explain a portion of the 8% decrease in the season-long stimulatory effect of elevated CO₂ under Low- compared with High-N under severe (1997) compared with moderate (1996) N stress (Figure 5) (*accept hypothesis 3*).

Because Rubisco is not usually catabolized for remobilization of N from leaves until grain filling (Simpson 1992; Simpson et al. 1992; Fisher 1993), any reduction in midday A (Figures 1 and 3a,b) that resulted from an increase in CO₂ under Low-N during vegetative growth, without a corresponding reduction in either midday g_s (Figures 2 and 3c,d) or midday C_i/C_a (Figure 3e,f), probably occurred because of enhanced N use efficiency for growth (Coleman et al. 1993; Rogers et al. 1993; Sage 1994, 1996; Drake et al. 1997) balanced with N allocated for energy

transduction (Osborne et al. 1997) and carboxylation capacity (Woodrow 1994; Webber et al. 1995; Osborne et al. 1998) (*accept hypothesis 4*).

Our results were based on the uppermost expanded sunlit leaf blade where light was not limiting. Consequently, we believe that no reallocation of N for energy transduction typical of a shade acclimation response occurred in these leaves (Long and Drake 1991; Evans 1993; Osborne et al. 1997). Any reductions in A, A' or A'' were more closely associated with the effects of elevated CO₂ and N stress on diminished carboxylation capacity (Adam et al. 2000), rather than changes in the diffusion conductance of CO₂. Hence, we believe that the magnitude of the difference in the stimulatory effect of elevated CO₂ on A (Figures 1 and 3a,b) and subsequent values of A' (Figure 4) and A'' (Figure 5) observed under High- compared with Low-N was large enough to support the premise that major alterations in the quantity of Rubisco occurred even in the uppermost expanded sunlit leaf. This decrease in carboxylation capacity in leaves of wheat constitutes an acclimatory response to rising levels of atmospheric CO₂ concentration under N-limited conditions (*accept hypothesis 5*).

Adam et al. (2000) reported that acclimation of the photosynthetic apparatus of wheat was dependent on atmospheric CO₂ concentration, N availability, and leaf position and age. Results reported herein suggest that elevated CO₂ and limited N supply caused an acclimation response for even the uppermost expanded sunlit leaf, especially, in later growth stages. Furthermore, based on biochemical results, reductions in carboxylation capacity because of elevated CO₂ and limited N supply were consistent with reductions in leaf net assimilation rates observed in this study (Figures 1, 3a,b, 4, and 5). A discrepancy exists, however, because elevated CO₂ stimulated net assimilation rate by 30% for individual leaves (Figure 1), but by only 13% for the whole canopy (Brooks et al. 2000). Comparisons between individual leaf and whole-canopy carbon uptake usually differ because they are based on leaf- and ground-area, respectively. Nevertheless, difference between individual leaf and whole-canopy carbon uptake can also be explained by differences in the effect of elevated CO₂ and either water or N stress on acclimation response within the canopy. For well-watered wheat grown with ample N supply, Osborne et al. (1998) reported a decrease in carboxylation capacity in leaves lower in the canopy. Elevated CO₂ also increased quantum efficiency of net assimilation, decreased the light compensation point, and decreased

photosynthetic capacity in leaves of a shade adapted forest under story plant, Indian strawberry (Osborne et al. 1997). In a companion study, Adam et al. (2000) reported that for the top three leaves of the wheat canopy N stress decreased carboxylation capacity with canopy depth. Also, elevated CO₂ can cause alterations in light (radiation use efficiency), temperature and vapor pressure gradients within the canopy that can affect both the magnitude and the direction of the photosynthetic response of individual leaves, thereby affecting whole-canopy carbon uptake (Long 1991).

Brooks et al. (2000) demonstrated that compensatory changes in stress driven morphological responses within the whole canopy could also mitigate the degree of the CO₂ and N treatment response observed at the biochemical and individual leaf levels. Nitrogen stress not only caused xeromorphic adaptations in leaf tissue which improved water status (Morgan 1986), but it also caused a more erect canopy structure (Araus et al. 1986a, 1993; Brooks et al. 2000). The more erect canopy architecture, commonly observed under N stress, would tend to increase light and temperature with canopy depth, which would alter water vapor pressure regimes of leaves within the canopy (Green 1987; Garcia et al. 1988). In contrast, the more planar canopy architecture, commonly observed for non-stressed wheat canopies, would lower light, and temperature with canopy depth, which could also alter water vapor pressure regimes. Compared with a more erect canopy, a more planar one is more likely to contribute to the observed acclimation response under elevated CO₂. Hence, a positive feedback between the compensatory effect of an increase in light and temperature, and alteration in water vapor pressure regimes for N-stressed canopies could have played a role in significantly mitigating the stimulation of individual leaf carbon gain by elevated CO₂. Further support of this feedback was obtained from results on both above- and below-ground quantities of net primary production (Pinter et al. 1996), which were in better agreement with whole canopy rather than individual leaf or biochemical-based carbon gain.

Conclusions

Our results demonstrate the inherent complexities in accounting for any acclimatory response of wheat to global change across biochemical (Adam et al. 2000), individual leaf (results reported herein) and whole canopy (Brooks et al. 2000) levels. Nevertheless, des-

pite any differences in the magnitude of the treatment response between individual leaf and whole canopy, the relative response in carbon gain was consistent across different scales. Hence, an acclimatory (*down-regulation*) response in the photosynthetic apparatus of field-grown wheat is anticipated in a future high-CO₂ world, but only if nitrogen is limited. Our results also demonstrate, however, that the stimulatory effect of a rise in atmospheric CO₂ on carbon gain in wheat can be maintained if nutrients such as nitrogen are in ample supply.

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